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Plant Growth Response to Several Allelopathic Chemicals[1]

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Abstract. **Salicylic acid, *p*-hydroxybenzoic acid, caffeine, hydroquinone, and umbelliferone were evaluated in the greenhouse for their effects on shoot dry-weight accumulation of several crop and weed species. With the exception of caffeine, all the chemicals reduced shoot growth in oats (*Avena sativa* L. 'Goodfield'). Chemicals applied preplant incorporated, preemergence, or postemergence were effective, depending upon the rate of chemical. When applied preplant incorporated at rates as high as 56.0 kg/ha, most of the chemicals reduced growth of corn (*Zea mays* L. 'B73 × Mo17'), soybean [*Glycine max* (L.) Merr. 'Corsoy'], velvetleaf (*Abutilon theophrasti* Medic.), redroot pigweed (*Amaranthus retroflexus* L.), and wild proso millet (*Panicum miliaceum* L.). Exceptions were caffeine on corn and soybean and hydroquinone on soybean. At 11.2 kg/ha the chemicals inhibited the weed species more than the crop species. Postemergence applications of caffeine and hydroquinone inhibited growth of the weed species more than the crop species. Hydroquinone at 1.1 kg/ha inhibited redroot pigweed, but rates as high as 11.2 kg/ha did not inhibit soybean. These experiments show that growth of agronomically important crops and weeds can be inhibited differentially by allelopathic chemicals.**

Additional index words. *Amaranthus retroflexus*, **allelopathy, caffeine, hydroquinone, phenolic acids.**

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INTRODUCTION

Plants produce a large number of compounds that have no obvious role in primary metabolism (21). One possible function for such secondary metabolites may be as protective agents in the plant (22, 23). Some of these metabolites are potent antibiotics and can aid in disease and insect resistance (23). Allelopathic effects of such compounds are important in interactions among plant species within both natural and agricultural ecosystems (15, 19, 24, 27). Soil sickness and accompanying reduced yields have often been attributed to inhibitory chemicals released from decomposing plant residues (2, 15). Allelopathic chemicals may inhibit crop growth directly, or may increase susceptibility of the crop to plant pathogens (1, 6, 16).

A wide variety of secondary plant metabolites can inhibit the growth of crop and weed species (5, 8, 14, 20). These allelopathic chemicals include phenolic acids, alkaloids, coumarins, and quinones (18). Representative chemicals in these groups are salicylic acid and *p*-hydroxybenzoic acid, caffeine, umbelliferone, and hydroquinone. Salicylic acid is a phenolic acid that has been identified in decomposing rye (*Secale cereale* L.) and corn residues (4) and soybean leaf tissue (10). Concentrations as low as 25 ppm are inhibitory to radicle growth in lettuce (*Lactuca sativa* L. 'Great Lakes') (4). *p*-Hydroxybenzoic acid is one of the most commonly identified benzoic acid derivatives involved in allelopathy (18). It has been found in corn, wheat (*Triticum aestivum* L.), sorghum [*Sorghum bicolor* (L.) Moench.], and oat residues, soybean leaf tissue, and cropped soils in many areas of the world (2, 10, 11, 26). It is thought to be most inhibitory in croplands in which localized areas of high concentration are found in association with plant residues (8, 25). Caffeine is an alkaloid found in higher plants and has been shown to inhibit seed germination (7, 18). Umbelliferone is a coumarin found in the resins of plants in the Umbelliferae. It may serve as a protective compound produced in response to infection by plant pathogens (12). Hydroquinone has been identified in the leachate of Eastwood manzanita (*Arctostaphylos glandulosa* Eastw.) (18). At 50 ppm it inhibited root growth of ripgut brome (*Bromus rigidus* Roth.) and wild oat (*Avena fatua* L.) (3).

It may be possible to use allelochemicals advantageously in crop production. Possibilities include breeding crop plants for increased production of chemicals that inhibit weed growth and producing natural herbicides from phytotoxic chemicals synthesized by plants (15, 20). For an allelochemical to be used as an herbicide, it would be advantageous for the compound to inhibit selectively weeds and not crops. However, few studies with allelopathic chemicals have compared their effects on more than one species.

The objective of this research was to compare the influence of the five representative allelopathic chemicals cited above on dry-matter accumulation by several crop and weed species. The five chemicals were chosen because they could be obtained inexpensively and in sufficient quantity. Emphasis was placed on determining the susceptibility of each species to the chemicals and comparing methods of application of the chemicals for phytotoxicity.

MATERIALS AND METHODS

General experimental procedures. Chemical treatments were applied to oats, corn, soybean, velvetleaf, redroot pigweed, and wild proso millet in the greenhouse. Chemicals were applied preplant incorporated, preemergence, and postemergence to simulate herbicide application in the field. Application rates were based on the surface area of 33 cm^2/946-ml plastic pot filled with silica sand (1500 g/pot). Approximately 5 ml of chemical solution were sprayed on each pot. Preplant incorporated treatments were mixed into the top 7.6 cm of sand in the pot. Preemergence and postemergence treatments were sprayed on the sand surface and plant foliage, respectively. Chemicals were dissolved in 25 to 95% (v/v) ethanol, depending on their solubility. Control plants were treated with ethanol at concentrations equal to the chemical treatments. A separate study showed that ethanol concentrations as high as 100% (v/v) did not affect oat growth.

To facilitate the collection of roots, most of the plants were grown from seed in silica sand. For one of the postemergence studies, seeds were planted in Plano silt loam (Typic Argiudoll, fine, silty, mixed mesic with 3.5 to 4.0% organic matter) and roots were not collected. After emergence, plants were thinned to either two or five plants/pot, depending on the experiment. Roots and shoots were harvested separately and dried for 24 h at 70 C. Plant dry weight per pot was determined and average weight per plant was calculated. Shoot dry weight is reported in this paper; results were comparable for shoots and roots.

All experiments were randomized complete block designs with three replications unless stated otherwise. All results were analyzed by analysis of variance. Means were separated by Duncan's multiple range test at the 95% confidence level. When the data ranged from near 0 to 100% of control, they were transformed to arcsine of the square root of the proportion (percentage/100) before the separation of means.

These studies were conducted between May 1980 and May 1981 in a temperature-controlled greenhouse maintained at 27 ± 3 C. Supplemental lighting from incandescent bulbs was provided for 16 h/day. In studies using silica sand, pots were surface-watered with one-half strength, modified Hoagland's nutrient solution (9). The pH of the nutrient solution was 6.5 and ranged from 6.0 to 6.5 upon addition of the test compounds. For the study in which plants were grown in soil, pots were surface-watered with tap water.

Preliminary studies. Each chemical was applied to oats preplant incorporated, preemergence at planting, or postemergence 7 days after planting (DAP) at rates of 11.2, 56.0, and 112.0 kg/ha. Plants were thinned to five/pot after emergence and harvested 21 DAP.

Preplant incorporated applications. Based on results of the preliminary experiments, preplant incorporated applications of chemicals at 11.2 and 56.0 kg/ha were selected for further studies. Each of the five chemicals was evaluated for inhibition of corn, soybean, velvetleaf, redroot pigweed, and wild proso millet growth. Weed species were harvested 28 DAP; crop species were harvested 14 DAP because of their rapid growth.

In another experiment, crop plants were harvested 14 and 28 DAP to compare the effects of the chemical treatments after different periods of growth. Oats, corn, and soybean were treated with 11.2 and 56.0 kg/ha of the three chemicals that were most inhibitory to these crops and were thinned to two plants/pot to allow for increased growth. This experiment consisted of four replications.

Weed susceptibility. Results with preplant incorporated chemicals indicated that the weed species were more susceptible than the crops to most of the chemicals at the low rate (11.2 kg/ha). Therefore, an experiment was conducted to determine which chemical had the greatest phytotoxic activity against each weed species. All five chemicals were applied preplant incorporated at a rate of 11.2 kg/ha to velvetleaf, redroot pigweed, and wild proso millet. Plants

Table 1. Effect of five naturally-occurring chemicals on oat shoot dry weight. Chemicals applied preplant incorporated, preemergence, or post-emergence at three rates.

Application		Chemical[a]				
Method	Rate	Salicylic acid	*p*-Hydroxybenzoic acid	Caffeine	Hydroquinone	Umbelliferone
	(kg/ha)	(mg/plant)				
Control	0.0	140.6a	117.8a	123.3ab	106.5ab	155.3a
Preplant incorporated	11.2	134.5a	98.4bcd	123.9ab	123.5a	146.5a
	56.0	40.4cd	83.5cd	108.2ab	96.3b	59.6d
	112.0	29.7d	56.2e	127.3ab	97.2b	25.3e
Preemergence	11.2	136.0a	82.5d	120.7ab	106.9ab	70.8d
	56.0	120.0a	110.3ab	144.0a	107.3ab	59.2d
	112.0	56.9bc	99.2bcd	121.9ab	110.0ab	51.6d
Postemergence	11.2	66.7b	90.2cd	111.5ab	100.6b	121.4b
	56.0	75.3b	91.6cd	86.7b	50.3c	94.9c
	112.0	41.0cd	101.3abc	88.8b	34.8c	61.7d

[a]Means within a column followed by the same letter are not significantly different at the 95% confidence level as determined by Duncan's multiple range test.

were thinned to five/pot after emergence and harvested 28 DAP.

Postemergence applications. In the preliminary studies, hydroquinone was inhibitory only when applied postemergence. Caffeine and hydroquinone were effective in reducing growth in the weed-susceptibility studies, but were less inhibitory to crop species. Therefore, effects of post-emergence applications of these two chemicals were studied at rates of 11.2 and 28.0 kg/ha on the crop and weed species. Weed species were planted 7 days earlier than crop species to allow for slower growth of the former. Chemical treatments were applied to all plants on the same day at 9 DAP for crops and 16 DAP for weeds. Plants were harvested 14 days after treatment.

In another study, lower rates of hydroquinone (1.1, 2.8, 5.6, and 11.2 kg/ha) were compared for inhibition of redroot pigweed and soybean plants grown in soil. Soybean plants were treated at initiation of expansion of the first trifoliate leaf. To compare susceptibility of redroot pigweed seedlings at different stages of growth, plants were treated when they had two to three, four to five, or seven to eight leaves. Plants were harvested 14 days after treatment.

RESULTS AND DISCUSSION

Preliminary studies. With the exception of caffeine, all the chemicals significantly reduced shoot dry weight of oats. The effectiveness of each method of application varied among chemicals. Oat growth was reduced significantly by salicylic acid, *p*-hydroxybenzoic acid, or umbelliferone applied preplant incorporated at rates of 56.0 or 112.0 kg/ha (Table 1). *p*-Hydroxybenzoic acid also reduced dry weight at the lowest rate of 11.2 kg/ha. Caffeine and hydroquinone did not inhibit growth at any rate when preplant incorporated. When the chemicals were applied preemergence, the highest rate of salicylic acid, the highest and lowest rates of *p*-hydroxybenzoic acid, and all rates of umbelliferone reduced growth. Caffeine and hydroquinone were not inhibitory at any rate. Postemergence application of salicylic acid or umbelliferone inhibited growth at all rates. Hydroquinone reduced dry weight when applied postemergence at the two highest rates, but caffeine did not inhibit at any rate.

In many previous studies, allelopathic effects were measured in response to chemicals at specific molar concentrations instead of rates in kilograms per hectare. The rates we used, when converted to molarities in solution in the pots, were approximately equivalent to 0.1 to 1 mM. Patterson (14) found that phenolic acids at concentrations of 1 mM reduced growth and physiological processes, including photosynthesis, of soybean. Rasmussen and Einhellig (17) reported inhibition of sorghum germination by two phenolic compounds at 10 mM. Growth and dry-weight accumulation of sorghum seedlings were inhibited at 5 and 0.5 mM, respectively (17). Although the rates we used are high in comparison to those recommended for most commercial herbicides, several phenolics have been extracted from different soils at concentrations as high as 49 μM in the soil solution (26). It is possible that even higher concentrations exist in localized regions of plant-residue accumulation in soil (13). It is also possible that these chemicals may be less active in soil because of adsorption to soil colloids and organic matter.

Preplant incorporated applications. When applied preplant incorporated, the chemicals inhibited growth of crop and weed species to different extents depending upon the species and the rate of application (Table 2). When applied at 56.0 kg/ha, salicylic and *p*-hydroxybenzoic acids and umbelliferone reduced shoot dry weight of all the crop and weed species. At 11.2 kg/ha, none of the chemicals inhibited soybean growth, but *p*-hydroxybenzoic acid and hydroquinone reduced corn dry weight slightly. Caffeine reduced growth of all three weeds at 11.2 kg/ha, but did not affect the growth of either crop even at 56.0 kg/ha. Hydroquinone was least

Table 2. Effect of five naturally-occurring chemicals applied preplant incorporated on shoot dry weight of five crop and weed species[a].

Application		Species[b]				
Rate	Chemical	Corn	Soybean	Velvetleaf	Redroot pigweed	Wild proso millet
(kg/ha)		(mg/plant)				
0.0	Control	109.2a	150.5a	49.1ab	11.9a	103.8a
11.2	Salicylic acid	100.1ab	145.0a	41.2bc	7.3b	73.6ab
	p-Hydroxybenzoic acid	90.2b	145.0a	38.1c	10.3a	49.1bc
	Caffeine	105.9ab	154.7a	25.5d	7.6b	58.0bc
	Hydroquinone	89.8b	148.4a	48.6ab	10.1a	75.2ab
	Umbelliferone	103.3ab	143.7a	35.9c	7.1b	68.2b
56.0	Salicylic acid	35.7c	114.6b	16.5de	2.8c	15.1d
	p-Hydroxybenzoic acid	39.8c	115.6b	19.6de	3.6c	9.4d
	Caffeine	95.7ab	154.1a	15.7e	2.6c	46.7bc
	Hydroquinone	91.8b	149.8a	52.6a	11.6a	53.3bc
	Umbelliferone	26.5c	113.5b	9.7e	1.7c	15.4cd

[a]Corn and soybeans were harvested 14 DAP; velvetleaf, redroot pigweed, and wild proso millet were harvested 28 DAP.

[b]Means within a column followed by the same letter are not significantly different at the 95% confidence level as determined by Duncan's multiple range test.

inhibitory; it reduced only corn growth at 11.2 kg/ha and both corn and wild proso millet growth at 56.0 kg/ha. At the low rate, weed species were more susceptible than crop species to these chemicals.

Because the two different growth periods (14 days for crops and 28 days for weeds) may have influenced the results in Table 2, we compared salicylic and *p*-hydroxybenzoic acids and umbelliferone for their effects on shoot dry weight of corn, soybean, and oats harvested both 14 and 28 DAP. At the low rate, salicylic acid and umbelliferone inhibited the growth of oats less at 28 DAP than at 14 DAP (Table 3). At the low rate, none of the chemical treatments reduced growth of corn or soybean at either time of harvest. The high rate of all three chemicals significantly reduced the dry weight of corn and oats at both harvest dates, but did not inhibit more at 28 DAP than at 14 DAP. The high rate of the chemicals appeared to reduce soybean growth more at 28 DAP than at 14 DAP. However, the cotyledons accounted for most of the weight of both control and treated plants at 14 DAP. At this age, treated soybean had no expanded leaves, whereas the first trifoliates were expanding on control plants. Weight of the dried cotyledons offset this difference, giving the false impression of no difference between controls and chemically-treated plants. By 28 DAP, the treated plants were recovering; the cotyledons had senesced, and thus weights were more representative

Table 3. Effect of three naturally-occurring chemicals applied preplant incorporated on shoot dry weight of three crop species harvested 14 and 28 DAP.

		Chemical[a], kg/ha					
		Salicylic acid		*p*-Hydroxybenzoic acid		Umbelliferone	
Harvest time	Species	11.2	56.0	11.2	56.0	11.2	56.0
(DAP)		(% of control)					
14	Corn	83abc	40d	92a	55b	95ab	51cd
	Soybean	89abc	77bc[b]	98a	92a[b]	92ab	83b[b]
	Oats	64cd	3f	99a	36c	64c	44d
28	Corn	103a	72bc	100a	51b	99a	61c
	Soybean	93ab	44d	97a	58b	93ab	40d
	Oats	94ab	14e	97a	51b	88ab	42d

[a]Means under the same chemical treatment followed by the same letter are not significantly different at the 95% confidence level as determined by Duncan's multiple range test performed on transformed data.

[b]Soybean cotyledons accounted for most of the weight of these plants. There were no expanded leaves. In control plants, the first trifoliates were beginning to expand.

Table 4. Effect on shoot dry weight of three weed species by five naturally-occurring chemicals applied preplant incorporated at 11.2 kg/ha.

Chemical	Species[a] Velvetleaf	Redroot pigweed	Wild proso millet
	(% of control)		
Control	100ab (34.8)[b]	100ab (18.8)	100ab (19.7)
Salicylic acid	90abc	58fg	85abc
p-Hydroxybenzoic acid	84bcd	49gh	103a
Caffeine	35h	40h	60efg
Hydroquinone	82cd	38h	69def
Umbelliferone	39h	63efg	78cde

[a]Means followed by the same letter are not significantly different at the 95% confidence level as determined by Duncan's multiple range test performed on transformed data.

[b]Values in parentheses are mean dry weights of the controls in mg/plant.

of the actual state of development of the plants. These results showed that at 28 DAP the injury by salicylic acid, *p*-hydroxybenzoic acid, and umbelliferone was no greater than at 14 DAP and was even less in several instances. Thus, if for Table 2 the crops had been harvested at 28 days as the weeds were, these chemicals may have shown even greater relative toxicity against weeds as compared to crop species. This selectivity might help crops establish a competitive advantage over weeds in the field.

Weed-susceptibility studies. Preplant-incorporated application of caffeine, hydroquinone, or umbelliferone at 11.2 kg/ha reduced the growth of all three weed species (Table 4); caffeine was the most inhibitory. The greatest reduction in growth resulted from treatment of velvetleaf with caffeine or umbelliferone, and redroot pigweed with *p*-hydroxybenzoic acid, caffeine, or hydroquinone. Only salicylic acid or *p*-hydroxybenzoic acid applied to velvetleaf or wild proso millet did not reduce growth.

Postemergence applications. Postemergence application of caffeine or hydroquinone significantly reduced growth of velvetleaf and wild proso millet (Table 5); however, this reduction did not seem to be greater than that caused by these chemicals when applied preplant incorporated. Redroot pigweed was the most susceptible of the plant species to postemergence application of the two compounds. The high rate (28.0 kg/ha) of caffeine and both rates of hydroquinone killed redroot pigweed within 3 days of application. At 11.2 kg/ha, hydroquinone was more effective in reducing redroot pigweed growth when applied postemergence (Table 5) rather than preplant incorporated (Table 4). On the other hand, hydroquinone was less inhibitory to corn when applied postemergence (Table 5) than preplant incorporated (Table 2).

Because redroot pigweed was very sensitive to postemergence treatments of hydroquinone, effects of lower rates of this chemical were compared on pigweed and soybean. Growth of soybeans treated at initiation of expansion of the first trifoliate leaf was not inhibited significantly by any of the rates from 1.1 to 11.2 kg/ha (Table 6). However, at the same rates hydroquinone inhibited redroot pigweed. The chemical killed or severely damaged the expanded redroot pigweed leaves. Ability of pigweed to recover depended on extent of damage to unexpanded leaves and the meristem. Susceptibility was greatest for plants treated at the two- to three-leaf stage. With those plants, each increase in rate of hydroquinone inhibited growth to a greater extent. At the four- to five-leaf stage only the highest rate inhibited growth more than the other three rates. At the seven- to eight-leaf stage, all four rates inhibited growth to the same degree.

Collectively, these results showed differential toxicity of the allelopathic chemicals among the species depending upon the rate and method of chemical application. Compared to commercially available herbicides, much higher rates of these chemicals were required to reduce plant growth. Thus, it is improbable that these chemicals would be useful as applied herbicides. However, it is probable that in agroecosystems these allelopathic chemicals could selectively inhibit weed species and not crop species. Such selective

Table 5. Effect of caffeine and hydroquinone applied postemergence on shoot dry weight of five crop and weed species.

Application Rate	Chemical	Species[a] Corn	Soybean	Velvetleaf	Redroot pigweed	Wild proso millet
(kg/ha)		(% control)				
0.0	Control	100ab (309.1)[b]	100ab (324.6)	100ab (42.8)	100ab (24.0)	100ab (19.1)
11.2	Caffeine	95abc	82a-d	84a-d	34g	103a
	Hydroquinone	85abc	80a-d	75a-e	0i	82a-d
28.0	Caffeine	72a-e	65cde	54def	0i	48efg
	Hydroquinone	69b-e	71a-e	40fg	0i	24h

[a]Means followed by the same letter are not significantly different at the 95% confidence level as determined by Duncan's multiple range test performed on transformed data.

[b]Values in parentheses are mean dry weights of the controls in mg/plant.

Table 6. Effect of hydroquinone applied postemergence on shoot dry weight of soil-grown redroot pigweed and soybean.

Application rate	Growth stage at treatment[a]			
	Soybean[b]	Redroot pigweed[b]		
	First trifoliate	2- to 3-leaf	4- to 5-leaf	7- to 8-leaf
(kg/ha)	(% control)			
Control	100a (314.8)[c]	100a (110.4)	100a (142.2)	100a (142.2)
1.1	104a	69b	58b	68b
2.8	99a	35c	55b	76b
5.6	101a	17d	57b	77b
11.2	106a	0e	29c	68b

[a]Soybean was treated 10 DAP. Redroot pigweed at the 2- to 3-leaf, 4- to 5-leaf, and 7- to 8-leaf stages were treated 7, 15, and 21 DAP, respectively.

[b]Means within a column followed by the same letter are not significantly different at the 95% confidence level as determined by Duncan's multiple range test performed on transformed data.

[c]Values in parentheses are mean dry weights of the control in mg/plant.

inhibition would depend upon concentration and distribution of these chemicals in the soil, as well as the crop and weed species present.

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